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National Food Safety Standard - Niacin

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FAIRS Subject Report

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Report Highlights:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Niacin and Niacinamide in Foods for Infants and Young Children, Raw Milk, and Dairy Products" as SPS/N/CHN/159. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Executive Summary:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Niacin and Niacinamide in Foods for Infants and Young Children, Raw Milk, and Dairy Products" as SPS/N/CHN/159. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Thanks go to the consortium of industry and 3rd country Embassies in Beijing for their assistance in translating and reviewing this standard.

This report contains an UNOFFICIAL translation of National Standard on Determination of Niacin and Niacinamide in Foods for Infants and Young Children, Raw Milk, and Dairy Products.

General Information:

BEGIN TRANSLATION

The National Standard of People's Republic of China

GB—××××

Determination of Niacin and Niacinamide in Foods for Infants and Young Children, Raw Milk, and Dairy Products

Draft for Comment

Issued on xx-xx-xxxx Implemented on xx-xx-xxxx

Issued by the Ministry of Health

of the People's Republic of China

1. Scope

This standard is formulated as a microbial method for determination of vitamin niacin and niacinamide.

This standard is applicable to the Determination of Niacin and Niacinamide in Foods for Infants and Young Children, Raw Milk, and Dairy Products.

2. Referenced normative documents

The following standards contain provisions, which through reference in this text, constitute provisions of this Standard. For dated references, subsequent amendments (exclude correction) to or revisions of any of these publications shall not apply to this Standard. All parties are subject to agreements based on this Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. For undated references the latest edition of the publication referred to

applies.

GB/T 6682 Water for analytical laboratory use—Specification and test methods

3. Principle

Vitamin niacin and niacinamide content of infant formula is estimated from acidimetric or densitometer response of *Lactobacillus plantarum* (ATCC 8014).

4. Reagent and culture medium

All reagents, without special specification, refers to analytic reagents; All experiment water, refers to level 2 water.

4.1. H_2SO_4 solution A: 10mol/L.

Pour the 95%-98%(v/v)concentrated sulfuric acid 280ml slowly in 600 water and stir. Then cool-off and dilute to 1000ml.

4.2. H_2SO_4 solution B: 1mol/L.

Dilute 100ml H_2SO_4 solution A with water to 1000ml.

4.3. NaOH solution: 150g/l

Weigh 150g NaOH in 1000ml beaker, dissolved by 400ml water. Cool-off to room temperature and dilute to 1000ml.

4.4. NaOH solution: 0.1mol/l

Weigh 4g NaOH (accurate 0.1mg) in 1000ml beaker, demarcate by potassium acid phthalate.

4.5. HCL solution: 0.1mol/l.

Dilute 3.65g hydrochloric acid to 1000ml with water.

4.6. Ethanol solution: 25% (v/v)

Dilute 250ml absolute ethanol to 1000ml with water.

4.7. Strain: *Lactobacillus plantarum* (ATCC 8014)

4.8. Culture medium:

4.8.1. Lactobacillus agar culture medium: peptonized milk 15g, yeast extract 5g, glucose 10g, tomato juice 100ml, monopotassium phosphate 2g, Poly-sorbose Monooleate 1g, agar 10g, add distilled water to total 1000ml, adjust PH to 6.8±0.2(25 ℃).

4.8.2. Lactobacillus broth culture medium: peptonized milk 15g, yeast extract 5g, glucose 10g, tomato juice 100ml, monopotassium phosphate 2g, Poly-sorbose Monooleate 1g, add distilled water to total 1000ml, adjust PH to 6.8±0.2 (25 ℃).

4.8.3. Vitamin niacin and niacinamide determine medium: Casamino Acid for vitamin determination 12g, glucose 40g, Sodium 20g, L-Cystine 0.4g, DL-Tryptophan 0.2g, adenine hydrochloride 20mg, guanine hydrochloride 20mg, uracil 20mg, thiamine hydrochloride 200μg, Calcium Pantothenate 200μg, pyridoxine hydrochloride 400μg, Riboflavin 400μg, β-amino acid 100μg, Biotin 0.8μg, dipotassium hydrogen phosphate 1g, potassium dihydrogen phosphate 1g, Magnesium sulfate 0.4g, sodium 20mg, Ammonium Sulfate Iron 20mg, manganese sulfate 20mg, add distilled water to total 1000ml, adjust PH to 6.7±0.2 (25 ℃).

(note: the commercial synthetic medium is better.)

4.9. Standard solution

4.9.1. Stock standard solution.—100 μg/mL.

Weigh dried niacin Reference Standard to 50–60 mg (accurate to 0.1mg) from phosphorus

pentoxide desiccator. Using 25% (v/v) Ethanol solution(4.6) volume to 100ml, store in a refrigerator.

4.9.2. Inter mediate standard solution.—10 μg/mL.

Accurately pipet 10 mL stock standard solution (4.9.1), into 100 mL volumetric flask, dilute to volume with 25% (v/v) Ethanol solution (4.6), store in a refrigerator.

4.10. Normal saline: Weigh 0.9g NaCL into 100ml volumetric flask, dilute to volume, and mix thoroughly. Dispense each tube 10ml, and plug the caps, sterilize 15min at 121 ℃. Prepare fresh weekly.

5. Apparatus

Common lab equipment and

5.1. Spectrophotometer.

5.3. pH meter

5.4 Shakers

5.5 Analytical Balance: resolution 0.1mg.

6. Determination

6.1 Preparation of strain

6.1.1 Transfer a pure (Lactobacillus plantarum) ATCC 8014 strain from strain culture medium to 3 Lactobacillus agar culture medium tube (4.8.1). Incubate 24h at 37 ℃. Subculture monthly, and store in a refrigerator as monthly tube. Then subculture a Lactobacillus agar culture medium tube from monthly tube as daily tube, incubate 24h at 37 ℃. Subculture 3 new tubes from monthly tube every month.

6.1.2 Inoculate a tube of Lactobacillus broth culture medium (4.8.2) from daily tube, and incubate 24h at 37 ℃. Centrifuge culture for 10minutes at 2000r/min under sterilized condition, then decant supernate. Re-suspend cells by 10ml normal saline (4.10) and centrifuge it again. Repeat above steps again. Then re-suspend cells by 10 normal saline (4.10), transfer 1ml suspension into 10ml normal saline (4.10), mix thoroughly.

6.1.3 The transmittance of the suspension (6.1.2) at 550nm texted by spectrophotometer with normal saline (4.10) as blank reference should between 60%-80%.

6.2 Preparation of sample:

Weigh 2g (accurate 0.1mg) solid sample or 5g (accurate 0.1mg) liquid sample(equivalently contain 1mg niacin) into 250 mL conical beaker. Dissolved with 20 mL 1mol/L H_2SO_4 solution B (4.2), and sterilize 30min at 121 ℃.Adjust PH to 6.0-6.5 with 150g/l NaOH solution(4.3). And adjust PH to 4.5 with 150g/l HCL solution (4.5). Dilute volume to 100ml with water. Filter the solution, pipet 25ml supernatant into 100ml beaker. Adjust PH to 6.8 with 0.1mol/L NaOH solution (4.4). Transfer into 250ml volumetric flask, and dilute to volume.

6.3 working standard solution: concentration of niacin 100ng/ml

Pipet 5.0mL intermediate standard solution (4.9.2), diluted to 500ml with water. Prepare fresh for each assay.

6.4 preparation of standard curve

Add distilled water, standard solution and medium in tubes according to the table1, and make triplet.

Table1:

Tube No:	1	2	3	4	5	6	7
Distilled water:(ml)	5	5	4	3	2	1	0
Standard solution#:(ml)	0	0	1	2	3	4	5
Medium:(ml)	5	5	5	5	5	5	5

6.5 assay solution:
Add distilled water, sample solution and medium into according to the table2, and make triplet.

Table2:

Tube No:	1	2	3	4
Distilled water :(ml)	4	3	2	1
Sample solution: (ml)	1	2	3	4
Medium:(ml)	5	5	5	5

6.6 sterilize

Sterilize all tubes 10min at 121 ℃ and cool-off rapidly to culture temperature, to formation of lightest color. Ensure the heating and cooling condition regular(bad impact may occur if too congest or too much tubes in the autoclave).

6.7 Inoculation

Sterile inoculate 50μl suspension to each tubes except standard No1. Plug the caps, mix well all tubes.

6.8 Incubation

6.8.1 Titrimetric method

Choose a selected temperature (±0.5 ℃) between 30-40 ℃, incubate 72h. Predict the growth situation through visually inspect each tubes: un-incubated tube should clear, the sample tubes and standard tubes should have gradual growth and free of other bacteria. If the tube is contaminated by other microorganism, the result is invalid.

6.8.2 Densitometer method

Choose a selected temperature (±0.5 ℃) between 30-40 ℃, incubate 16-24h. Follow other step from 6.8.1.

6.9 assay

6.9.1 Titrimetric method

Titrate contents of each tube with NaOH solution (4.5), using bromthymol blue indicator, or using pH meter to pH 6.8. Disregard results of assay if titer of inoculated blank is more than 1.5mL greater than titer of un-inoculated blank. Titer at 5.0mL level of standard solution should be 8–12mL.

6.9.2 Densitometer method

Choose a suit parameter, assay the maximum concentration tube with using a blank inoculate tube as reference at 550nm. And assay this tube again after 2h. If the difference between this two result is ≤2%, that mean you can take out all the tubes and assay them.

6.10 draw standard curve

According to the microorganism growth characteristic of logarithmic phase and plateau phase, draw 2 sect of logarithmic curve. With the value of niacin in standard solution as X-axis, the value of densitometer (PH) as Y-axis, draw standard curve. As far as possible line through the middle of two discrete points and smooth the standard curve.

Quantitative determine vitamin of each concentration of assay solution. Abandon the absorption value

less than 0.5ml standard solution or high than 4.5ml standard solution.
For each concentration of assay solution, calculate the concentration of folic acid per ml. Calculate the average value, and each concentration assay solution value should not exceed the average ± 15%. If calculable value you received is less than 2 / 3 of total tubes, must be redone; If calculable value is more than 2 / 3 of total tubes, you can calculate content of samples according to the average of the value.

7. Calculations and indication
Content of niacin in sample according to this formula:

$X(\mu g/100g) = Cx/m \times F/1000 \times 100$

Cx = average value of niacin check from standard curve, μg;
F = dilution factor based on preparation of sample
m = test portion weight or volume, g or mL
100= conversion to per 100g
1000= conversion from ng to μg.
The result indicated with average of two separate calculation, and keep to one decimal.

8. Allowable error
The difference between the values of the twice tests to the same sample should ≤10%.

Method Ⅱ HPLC Method

9. Principles

After extracted with hot water and de-proteinized by acid, sample is separated by C-18 chromatogram column and determinated with a UV detector.

10. Material and Regents

If no special illumination, all reagents mentioned in this method are AR grade and water is grade Ⅲ water regulated in GB/T 6682.

10.1 α-amylase, enzymatic activity ≥ 1.5U/mg
10.2 Hydrochloric acid solution, c (HCl) = 5.0mol/L
10.3 Sodium hydroxide, c (NaOH) = 5.0mol/L
10.4 perchloric acid 60%V/V
10.5 Anhydrous Methanol, HPLC grade
10.6 Isopropyl, HPLC grade
10.7 Octane Sodium sulphonate
10.8 Standard Solution
10.8.1
Niacin and Niacinamide standard stock solution, the concentration is 100μg/mL.
Accurately weigh about 10.0mg of Niacin and Niacinamide standard material respectively to 100mL

volumetric flask, dissolved with water, and add water to the mark.
10.8.2
Niacin and Niacinamide standard working solution, the concentration is 5μg/mL
Respectively pipette 5ml of above-mentioned standard stock solution to 100mL volumetric flask, add water to the mark (both concentration are 5μg/mL).

11. Instruments and Equipment

The following instruments and equipments are common used in laboratory:
11.1 HPLC, equipped with UV detector
11.2 PH meter
11.3 Ultrasonator
11.4 Analytical balance, accurate to 0.1mg

12. Analysis procedure

12.1 Sample Pretreatment
12.1.1 Amylun Sample
For solid sample, weigh about 5.0g; for liquid sample should mix equality, weigh about 20g(accurate to 0.1mg), to a 150mL conical flask, add about 0.5g α-amylase, then add 25mL of 45-50 ℃ water and mix thoroughly. Fill nitrogen to the conical flask and seal it, placed it into 50-60 ℃ incubator for 30min, then cool it to room temperature.
12.1.2 No amylun Sample
For solid sample, weigh about 5.0g; for liquid sample should mix equality, weigh about 20g (accurate to 0.1mg), to a 150mL conical flask, add 25mL of 45-50 ℃ water and mix to dissolve thoroughly. Let it stand to room temperature.
12.1.3
Extraction: place above-mentioned conical flask to ultrasonator for 10min
12.1.4 Sedimentation and Constant Volume:
after the sample solution cooling to room temperature, adjust PH of it to 1.70 with hydrochloric acid solution(10.2), let it stand for 2min, then adjust pH of it to 4.50 with sodium hydroxide(10.3). Transfer the sample solution into a 50mL volumetric flask; wash the conical flask with distilled water repeatedly and combine the washing liquid to the 50mL volumetric flask then add water to the mark. Filter the solution through filter paper. Again filter the solution through 0.45μm membrane filter and collect the filtrate as injection sample.
12.2 Reference Chromatography Parameter
Column: C18, 50×4.6 mm, 5μm film, or equivalent
UV detector wavelength: 261nm
Inject volume: 10uL
Oven temperature: 25 ℃
Flow rate: 1.00mL/min
Mobile phase: 7.0 %(V/V) methanol, 2.0 %(V/V) isopropyl, 1g/L octane Sodium sulphonate, adjust pH of the mobile phase to 2.10 with perchloric acid and filter it through 0.45μm membrane filter.

12.3 Quantitative analysis (external standard method)
Inject a certain volume of standard working solution to HPLC to get area (or height) Ai of peak of compound i; Inject equal volume of sample solution to HPLC and get area (or height) Bi of peak of compound i.

13 Calculation and Expression

The content X of Niacin in sample solution, expressed by mass fraction milligram per hundred grams (mg/100g), calculated using formula (2):

$X = X1 + X2$…… （2）

In this formula,

X – The content of Vitamin PP in sample, the unit is milligram per hundred grams (mg/100g)

X_1 –The content of Niacin in sample, the unit is milligram per hundred grams (mg/100g)

X_2 –The content of Niacinamide in sample, the unit is milligram per hundred grams (mg/100g)

There into:

$$X_{1或2} = \frac{B_i \times C_s \times 100}{m \times A_i \times 1000} \quad \text{……} \quad (3)$$

In this formula,

$X_{1\ or\ 2}$ ——The content of Niacin or Niacinamide in sample, the unit is milligram per hundred grams (mg/100g)

m——Sample weight, the unit is gram（g）;

A_i ——the peak area (or height) of standard working solution gotten from 12.3;

B_i —— the peak area (or height) of sample solution gotten from 12.3;

c_s——the concentration of Niacin or Niacinamide in standard working solution, the unit is microgram per milliliter (μg/mL）;

V——the volume of sample solution, the unit is milliliter (mL).

The result is the arithmetical mean of two independent tests, and reserved two decimal digits.

14 Precision

Absolute difference of two independent test results should not exceed 10% of arithmetical mean under the repeated test condition.